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Title: PM10 size distribution of metals and environmental-sanitary risk analysis in the city of Torino

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Section/Category: Environmental Toxicology and Risk Assessment

Keywords: PM10; metals; health-sanitary risk analysis

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Response to Reviewers: REVIEWER COMMENTS

Reviewer #1:

Review of the manuscript CHEM31198 entitled "PM10 size distribution of metals and environmental-sanitary risk analysis in the city of Torino". In this paper the authors investigated the metal distribution in different fraction of PM samples collected in a north Italian city (Torino) evaluating the risk associated to airborne PM10 metals by quantitative risk assessment (QRA). The subject of the paper is interesting, in particular considering that there are few works that associate the PM metal distribution in different fraction to health effects using QRA.
It is not within my competence but English grammar should be correct all over the manuscript.

The work is well organized but some considerations about the manuscript are reported below:
Page 3 line 55 better clarify the second bullet point
Page 3 line 56 add "some" after "risk for"
Page 3 line 59 delete "Particulate Matter" or "PM10" and add another key word
Page 4 line 95 add other references (e.g. Bonetta et al., 2009)
Page 5 line 121-125 some repetition appears in the , please read better and rewrite
Page 6 line 131 add some example of fields of interest
Page 6 line 142 delete "only"
Page 6 line 152 check the word "weighing"
Page 7 line 158 delete the word "starting"
Page 7 line 161 detail the technique used (ICP? or others ?)
Page 7 line 171 detail the different PM fraction considered
Page 7 line 174 explain why only inhalation route was considered
Page 7 line 177 add after "calculated" the sentence "…,where possible,…"
Page 8 line 182-183 moves this sentence after "…to be without effect.." page 7 line 181 and add the corresponding equation

Page 8 line 191 explain better "one field"
Page 8 line 192 add "…,where possible,…" after "for each contaminant"
Page 8 line 193-195 better explain adding the corresponding equation
Page 8 line 202-204 moves the sentence after "for each contaminant" line 192
Page 8 line 204 check the word ingestion and change with inhalation
Page 8 line 205-207 add some references
Page 9 line 210 detail better the different fraction investigated (eight and three)
Page 9 line 220 add "Italian regulation" after "set by"
Page 9 line 230 add "Italian regulation" after "set by"
Page 9 line 230-231 check the sentence
Page 10 line 242 add (Table 3) at the end of the sentence
Page 10 line 247 moves "Figure 1" after "fine PM1" line 244
Page 10 line 247 explain and detail the trend of metal distribution in the different fraction
Page 10 line 257 add the range of risk revealed
Page 11 line 260 check this sentence because a clear differences between adult and child is not showed in the figure
Page 11 line 262-263 moves this sentence after "Cd" line 263
Page 11 line 271 add the value of risk calculated
Page 11 line 282 add "using QRA"
Page 12 line 290-300 this period is too long
Page 12 line 301 add "toxic or" after "potential"
Page 13 line add consideration regarding the risk associated to the metal presence in the different fractions

Table 1 add reference
Table 2 change ISPLES with ISPESL and SF with CSF
Table 3 change "expressed as ng/m3" with "expressed as mean ng/m3" and moves the sentence "in particular….textured filter" in the results section.

Reviewer #2:

The manuscript "PM10 size distribution of metals and environmental-1 sanitary risk analysis in the city of Torino" by Romanazzi et al. aims to evaluate the levels and distribution of 16 particulate-bound metals and to assess the respective non-carcinogenic and carcinogenic risks.
The manuscript is clearly written. The number of figures and tables are adequate and well organized. The used techniques are satisfactory but detailed description concerning the analytical process is missing. Although the obtained results might be interesting to readers of Chemosphere I have two major comments:

1. This manuscript reports findings based on 3-day PM sampling at 1 site. Although 7- stage impactor was used (i.e. total of 24 samples) this seems as rather limited set of data /short period. Furthermore, the obtained levels of PM metals are then compared with annual target values. I have doubts about relevance of this information in view of 3-day sample collection during 1 season at one site only. In addition for international readers it will be interesting to compare obtained levels of metals (and the respective risks) with European or USEPA guidelines rather than Italian national limits/risks guidelines.

2. Authors estimated risks due to exposure to particulate-bounds metals, i.e. inhalation exposure by USEPA methodology. However, their calculations are performed using values for ingestions (i.e. oral exposure). I strongly advice authors to revise (and to correct) their approach. In the comments below I attach the link for respective USEPA methodology. In addition, the selection of some critical

parameters (body weight, inhalation rates, period of daily outdoor exposure) needs clarification/justification. For that authors should consult the respective USEPA reports (please see in specific comments). The discussion should also include more detailed information/comparisons of risks of both age categories (children versus adults)

Further comments are listed in the section below which I hope to improve author their work

INTRODUCTION
Line 117-120: Information concerning EU legislation on carcinogenic metals (As, Cd, Ni) in ambient air should be completed: indicated targets are annual ones and determined in PM10.
Line 121-132: rephrase this section and clearly state the objectives of this work. Why information about "historical evolution of size fractioned PM10 evolution of the ". Is it relevant to this work? Please clarify/rephrase
Line 128-131: Information about importance of risk assessment should be placed in the previously in introduction and not in the objectives.
Please correct references within whole manuscript once space appears before comma ("al. ,")

MATERIALS AND METHODS
Section 2.1:
Sampling site and its characteristic should be better described: define site (urban, background) and it major influences (traffic, industrial, etc.). What were the surroundings of sampling site?
Section 2.2:
Information on quality control/assurance of analytical process should be introduced. LODs/LOQs are missing too.
Section 2.3:
1. The non-carcinogenic and carcinogenic risks to particulate trace elements were calculated according to the USEPA methodology. Information concerning USEPA risk guidelines should be therefore included (THQ less than 1; for CR considers risk range 10-6 to 10-4: values below 10-6 for individual chemicals and pathways will cause negligible cancer risks but caution is recommended to ensure that the cumulative cancer risk for all potential carcinogenic contaminants does not have a residual cancer risk exceeding 10 −4).
2. Authors should justify the selected values for the following parameters in Table 1: BW, IR, Bo. For example inhalation rate of 0.9 L/min correspond to what level of physical activity? Please see USEPA materials concerning the respective information for adult and children, respectively:
USEPA Exposure Factors Handbook: http://www.epa.gov/ncea/efh/pdfs/efh-complete.pdf
Child-Specific Exposure Factors Handbook:
http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=199243
In addition, EFg of 6h/day (25%) for outdoor exposure seems rather high once people spend indoors typically 80-95%. Once again authors should justify/correct the selected value.
3. Finally, authors estimate risks due to exposure to metals in PM10 in ambient air. Assumingly these are inhalation risks but the THQ and CR calculation were done using reference dose (RfD) and cancer slope (CSF) for ingestion exposure. It is not clear to me why authors did not use the USEPA methodology for inhalation exposure (Chronic Inhalation Reference Concentration RfC (mg/m3); Chronic Inhalation Unit Risk IUR (µg/m3)-1 (THQ, TR). The available information can be found here: (section 4.9.1-2): http://www.epa.gov/reg3hwmd/risk/human/rb-concentration_table/usersguide.htm
I think that their approach needs major revisions.
4. Table 2: Reference [17]? Missing in the list of references (ISS-ISPESL).It is difficukt to find/confirm data in Italian database. Furthermore, once USEPA approach is used, RfD and CSF referred by USEPA should be use: please consult the following link for Regional Screening Level (RSL) Summary Table:
http://www.epa.gov/reg3hwmd/risk/human/rb-concentration_table/Generic_Tables/docs/master_sl_table_run_NOV2013.pdf

RESULT
Lines 222-233 (section 3.1) should be enhanced: authors compared average metals concentrations of 3 days measurements with annual targets. In addition, according to the statement in lines 140-142 the measurements were performed during winter season with greater exposure i.e. metals levels in ambient air; the EU target values might not be exceeded during other seasons.
Lines 264-265 This statement is obvious once PM10 contains both fractions. Remove/change.
Lines 265-266: Considering different deposition places of both fractions the statement "indeed total PM10……" is not correct. I am not sure what authors want to state. Furthermore according to Figure 1 most of the some metals (are predominantly (up to 70%) found in fine fraction.

DISCUSSION
Line 288-300 this section should be placed in introduction
Line 301: Mn causes carcinogenic risks…. CR risk assessment of Mn was not performed.
Authors should add discussion on different species metals and potential impacts. Finally, I believe that changes in calculations of THQ, CR using IUR, RfC will cause major changes/alterations which should be reflected in results/discussion.

RESPONSE TO REVIEWERS COMMENTS

Response to Reviewer #1 comments:

We thank the reviewer for the suggested amendments.
All specific corrections have been answered and are highlighted in yellow in the revised manuscript.

We clarified the second bullet point.
We added, deleted, clarified and rewrote all the reviewer #1 suggested.
We added some example of fields of interest at lines 135-137.
The technique utilized was “inductively coupled plasma - mass spectrometer”. It is now specified in the main text; globally more details concerning this method were provide (lines 170-185).
We detailed the different PM fraction considered (already reported in Section 2.1) in the main text.
A sentence was added and highlighted in the main text (lines 195-196) to explain why only inhalation route was considered.
We changed ingestion with inhalation all over the text, it was an authors mistake.
We substituted the Italian reference about legislation with the European Directive one (2008/50/EC) all over the manuscript.
We added two descriptive sentences at lines 297-299 and 302-303 to explain and detail the trend of metal distribution in the different fraction
We add the THQ and CR ranges modifying the sentences at lines 311-317 adding the range of risk revealed
The little CR difference between adult and child was not clear as suggested by the reviewer#1 in figure 3 because of the log scale, but adding the ranges in the previous sentence it is explained.
For Cd we add the THQ and CR ranges at lines 329-330.
Done, as required, at line 342, but we use “ by means of environmental-sanitary risk assessment” in order to be consistent.
We added consideration regarding the risk associated to the metal presence in the different fractions at lines 345-348.
In Table 2 (past Table 1) we added references: USEPA 1991, USEPA 2009 and USEPA 2011.

In Table 1 (past Table 2) we changed reference with one updated from USEPA (USEPA November 2013). New calculations were performed according with these new values.
In Table 3 we change what the reviewer suggested.

Response to Reviewer #2 comments:

We thank the reviewer for the suggested amendments.
All specific corrections have been answered and are highlighted in yellow in the revised manuscript).

Major Comments :
1.
Concerning the European guidelines, the D.Lgs 155, 2010 mentioned as reference represents the Italian transposition of the European Directive 2008/50/EC: we have replaced the Italian transposition with the European Directive along all the manuscript, in order to provide to international readers greater opportunity for comparison.
Concerning the 3-day PM sampling at one site, actually the sampling occurred during three autumn/winter days and this choice is due to different factors: - during this period the emission sources of atmospheric pollutants are more numerous and intense; -in the same period some atmospheric phenomena occur, such as thermal inversions, that can lead to a greater exposure levels for the population. These two factors are very important because in view of a risk analysis we looked the worst pollution conditions under the principle of maximum precaution.
Concerning the comparison with metals annual target values, we are aware of the discrepancy but it was only to provide the readers with a comparison, for this reason we underlined this at lines 276-277.

2.
About inhalation and ingestion routes, it was a spelling mistake. Ingestion clearly was not adequate, the used approach and values were referred to inhalation: we have corrected all over the main text (Also the other reviewer underlined the mistake!).
The methodology procedure for the estimation of inhalation exposure was detailed in USEPA 1989, USEPA 2009 –Part F and in USEPA 2011 and new details concerning the risk analysis calculation were added in Material and Methods Section (lines 231-250), according with USEPA Method. Default parameters used in the calculations of risk analysis are now detailed in Table 2. Further argumentation about your comment are present in Materials & Methods – Section 2.3.
About the comparisons of risks of both children and adults we highlighted this aspect at lines 312-317 and in the abstract at lies 37-38; we also add this consideration at lines 368-372.

Further comments:

INTRODUCTION
Information concerning EU legislation on carcinogenic metals (As, Cd, Ni) in ambient air were added at lines 128-130.

We rephrased the section and clearly stated the objectives of the work. In particular the historical evaluation based on a previous study on the same area (Casazza 2013) is fundamental since the change in the emission sources examined in that occasion is related to a different deposition of metals (and other pollutants not here investigated) on different fractions of PM10 (lines 138-143).

We added a brief comment about the importance of risk assessment in the introduction (lines 133-137).

The references has been revised for editing.

MATERIALS AND METHODS
Section 2.1:
Sampling site and its characteristic are highlighted at lines 162-164.
Section 2.2:
Details concerning quality control and LOD/LOQ were added and highlighted in the Materials and Methods Section (lines 188-191).
Section 2.3:
1.
Information concerning non-carcinogenic and carcinogenic risks guidelines are highlighted at lines 210-214 and lines 221-229 respectively.

2.
Simplified information were embedded in the caption of Table 1, now named Table 2; the table now receive the English acronyms of singular parameters, as well as the correct references (USEPA 1991, USEPA 2009 - Part F and USEPA 2011). The inhalation rates (IR) were 0,9 m3/h for adult and 0,7 m3/h for children considering male and female combined for sedentary and light activity (USEPA 2011). From "Standard Default Exposure Factors" (USEPA 1991) and from USEPA 2009 Part F, Exposure Frequency (EF), Exposure Duration (ED) and Body Weight (BW) parameters were inferred, taking into account the "Inhalation of contaminant" as exposure pathway in a "residential scenario". An example of a residential scenario could consist of inhalation exposure for up to 24 hours per day, up to 350 days per year for 6 to 24 years for child and adult respectively (USEPA 2009 - Part F).
The methodology procedure for the estimation of inhalation exposure was detailed in USEPA 2009 – Part F Document.
Concerning the exposure parameters related to EFg value (now daily Exposure Time= ET), the USEPA 1991 Document indicates "24 hours, the whole day" as "resident air exposure time" and then setting in according with time spent in a specific site. Actually, in the light of your suggestions, an ED value of 6 hours seems rather high, thus as recommended by USEPA table of time spent outdoor (mean of 289 min/day for maximum age range considered) and according with lower limit indicated by you (80-95% time spent indoor), we have correct it considering 5 hours as ED value. Calculation were again performed.

3.
About inhalation and ingestion routes, it was a spelling mistake (your comment n°2)
In the light of the suggestions, we have checked the USEPA methodology and new calculations were performed, taking into account the most recent USEPA update (November 2013) concerning RfC and IUR values, as added in the manuscript (page 9, lines 231-242).

4.
"Table 2" is now entitled "Table 1"; here the updated values from USEPA 2013 updated Table were reported as you suggested, and new specifications about it were provided in the text in the Material and Methods Section.

RESULT
section 3.1
We added a sentence at lines 276-277 to better explain the considerations about PM and metals concentration .
We removed the sentence about the PM10 inhalation and we improved the text at lines 300-303 as suggested.

DISCUSSION

We moved the indicated sentence in the introduction (lines 111-117).
About Mn it was a mistake so we added toxic referred to Mn (lines 349-350). We also discussed about different species metals and potential impacts (lines 350-362).

UNIVERSITY OF TORINO, ITALY
Department of Public Health and Pediatrics
Hygiene Division - Via Santena 5 bis
10126 TORINO, Italy

December 13th, 2013

Dear Editor,

We are sending the manuscript "*PM10 size distribution of metals and environmental-sanitary risk analysis in the city of Torino*" by Valeria Romanazzi, Marco Casazza, Mery Malandrino, Valter Maurino, Angelo Piano, Tiziana Schilirò*, Giorgio Gilli for possible publication on *Chemosphere.*

Referring to the biomonitoring of chemicals related to adverse health effects, the current study is aimed to calculate the environmental-sanitary risk linked to exposure to airborne PM10 metals. Metals PM10 size distribution analysis was carried out in a central site of Torino city-Italy. While the concentrations of all the sampled metals appeared to be under control, their presence in the different PM10 fractions and their sanitary risk provided indications related to the body districts potentially in contact with these substances.

The manuscript has been seen and approved by all co-authors. I confirm that neither the manuscript nor any part of it has been published or is under consideration for publication elsewhere.

Lastly, all authors declare no conflicting interests.

Hoping that the manuscript may fulfil the scientific standards of ***Chemosphere,*** my best regards.

Dr. Tiziana Schilirò

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e-mail: tiziana.schiliro@unito.it

HIGHLIGHTS:

- Traces metals are differently distributed among the PM10 fractions
- The metals distribution in PM fractions can be useful for sanitary risk assessment
- Sanitary risk assessment shows potential risks for exposure to some metals on PM

***Revision Notes**

Click here to download Revision Notes: Detailed Responses to ReviewersRevision notes.docx

Dear Editor,

please find enclosed the revised manuscript "*PM10 size distribution of metals and environmental-sanitary risk analysis in the city of Torino*" - CHEM31198 - by Valeria Romanazzi, Marco Casazza, Mery Malandrino, Valter Maurino, Angelo Piano, Tiziana Schilirò* and Giorgio Gilli.

We have answered to all the Reviewers' comments, in particular relevant changes have been highlighted in yellow all over the enclosed text. Finally, our comments to Reviewers have been reported as follows.

Best regards,

Tiziana Schilirò and Co-authors.

Reviewer #1

We thank the reviewer for the suggested amendments. All specific corrections have been answered and are listed in the original reviewer text below.

- *"It is not within my competence but English grammar should be correct all over the manuscript"*

The manuscript was carefully revised and corrected by a English native speaker and now it appears in a better grammatical form.

Other comments:

- *Page 3 line 55 better clarify the second bullet point*

  Ok, we clarified the second bullet point
- *Page 3 line 56 add "some" after "risk for"*

  Done, as you required.
- *Page 3 line 59 delete "Particulate Matter" or "PM10" and add another key word*

  Done, as you required.
- *Page 4 line 95 add other references (e.g. Bonetta et al., 2009)*

  Done, as you required.
- *Page 5 line 121-125 some repetition appears in the , please read better and rewrite*

  Done. In particular a repeated sentence was deleted
- *Page 6 line 131 add some example of fields of interest*

  Done; the added specifications were at lines 135-137.
- *Page 6 line 142 delete "only"*

  Done, as you required.

- *Page 6 line 152 check the word "weighing"*

  We have checked it, but appears right (= to weigh the filters)
- *Page 7 line 158 delete the word "starting"*

  Done, as you required.
- *Page 7 line 161 detail the technique used (ICP? or others ?)*

  Yes, the technique is "*inductively coupled plasma - mass spectrometer"*. It is now specified in the main text; globally more details concerning this method were provide, as you can see in the highlighted text (lines 170-185).
- *Page 7 line 171 detail the different PM fraction considered*

  Done. Details (already reported in Section 2.1) were added and highlighted in the main text
- *Page 7 line 174 explain why only inhalation route was considered*

  Done. A sentence was added and highlighted in the main text (lines 195-196).
- *Page 7 line 177 add after "calculated" the sentence "…,where possible,…"*

  Done, we add it at pag. 9 line 232.
- *Page 8 line 182-183 moves this sentence after "…to be without effect.." page 7 line 181 and add the corresponding equation*

  Done, as you required.
- *Page 8 line 191 explain better "one field"*

  Done, as you required.
- *Page 8 line 192 add "…,where possible,…" after "for each contaminant"*

  Done, we add it at pag. 9 line 232.
- *Page 8 line 193-195 better explain adding the corresponding equation*

  Done, as you required at line 245.
- *Page 8 line 202-204 moves the sentence after "for each contaminant" line 192*

  Done, as you required.
- *Page 8 line 204 check the word ingestion and change with inhalation*

  Done, as you required, thank you it was inhalation.
- *Page 8 line 205-207 add some references*

  Done, as you required.
- *Page 9 line 210 detail better the different fraction investigated (eight and three)*

  Done, as you required.
- *Page 9 line 220 add "Italian regulation" after "set by"*
- *Page 9 line 230 add "Italian regulation" after "set by"*

Done, as you required, but we add for the reference European Directive 2008/50/EC all over the manuscript.

- *Page 9 line 230-231 check the sentence*

  We have improved the sentence.

- *Page 10 line 242 add (Table 3) at the end of the sentence*

  At line 247 “Table 3” it was already present.

- *Page 10 line 247 moves "Figure 1" after "fine PM1" line 244*

  Done, as you required.

- *Page 10 line 247 explain and detail the trend of metal distribution in the different fraction*

  Done, as you required, we add two descriptive sentences at lines 297-299 and 302-303.

- *Page 10 line 257 add the range of risk revealed*

  Done, as you required, we add the THQ and CR ranges modifying the sentences at lines 311-317.

- *Page 11 line 260 check this sentence because a clear differences between adult and child is not showed in the figure.*

  Done, as you required: really the little CR difference between adult and child is not clear in figure 3 because of the log scale, but adding the ranges in the previous sentence it is explained.

- *Page 11 line 262-263 moves this sentence after "Cd" line 263*

  Thank you for the observation, we clarified the sentence.

- *Page 11 line 271 add the value of risk calculated*

  Done, as you required at lines 329-330.

- *Page 11 line 282 add "using QRA"*

  Done, as required, at line 342, but we use “ by means of environmental-sanitary risk assessment” in order to be consistent.

- *Page 12 line 290-300 this period is too long*

  Done, we modified the sentences.

- *Page 12 line 301 add "toxic or" after "potential"*

  Done, as required.

- *Page 13 line add consideration regarding the risk associated to the metal presence in the different fractions*

  We highlighted these considerations at lines 345-348.

- *Table 1 add reference*

  Now Table 2; done, we added USEPA 1991, USEPA 2009 and USEPA 2011 as references.

- *Table 2 change ISPLES with ISPESL and SF with CSF*

Now Table 1. Done, we changed that reference with one updated from USEPA (USEPA November 2013). New calculations were performed according with these new values.

- *Table 3 change "expressed as ng/m3" with "expressed as mean ng/m3" and moves the sentence "in particular….textured filter" in the results section.*

  Done as required.

Reviewer #2

We thank the reviewer for the suggested amendments. All specific corrections have been answered and are listed in the original reviewer text below.

Major Comments :

- *"1- This manuscript reports findings based on 3-day PM sampling at 1 site. Although 7- stage impactor was used (i.e. total of 24 samples) this seems as rather limited set of data /short period. Furthermore, the obtained levels of PM metals are then compared with annual target values. I have doubts about relevance of this information in view of 3-day sample collection during 1 season at one site only. In addition for international readers it will be interesting to compare obtained levels of metals (and the respective risks) with European or USEPA guidelines rather than Italian national limits/risks guidelines".*

  Concerning the European guidelines, the D.Lgs 155, 2010 mentioned as reference represents the Italian transposition of the European Directive 2008/50/EC: we have replaced the Italian transposition with the European Directive along all the manuscript, in order to provide to international readers greater opportunity for comparison.

  Concerning the 3-day PM sampling at one site, actually the sampling occurred during three autumn/winter days and this choice is due to different factors: - during this period the emission sources of atmospheric pollutants are more numerous and intense; -in the same period some atmospheric phenomena occur, such as thermal inversions, that can lead to a greater exposure levels for the population. These two factors are very important because in view of a risk analysis we looked the worst pollution conditions under the principle of maximum precaution.

  Concerning the comparison with metals annual target values, we are aware of the discrepancy but it was only to provide the readers with a comparison, for this reason we underlined this at lines 276-277.

- *"2- Authors estimated risks due to exposure to particulate-bounds metals, i.e. inhalation exposure by USEPA methodology. However, their calculations are performed using values for ingestions (i.e. oral exposure). I strongly advice authors to revise (and to correct) their approach. In the comments below I attach the link for respective USEPA methodology. In addition, the selection of some critical parameters*

(body weight, inhalation rates, period of daily outdoor exposure) needs clarification/justification. For that authors should consult the respective USEPA reports (please see in specific comments). The discussion should also include more detailed information/comparisons of risks of both age categories (children versus adults)".

Thank you for the observation. It was a spelling mistake. Ingestion clearly was not adequate, the used approach and values were referred to inhalation: we have corrected all over the main text (Also the other reviewer underlined the mistake!).

The methodology procedure for the estimation of inhalation exposure was detailed in USEPA 1989, USEPA 2009 –Part F and in USEPA 2011 and new details concerning the risk analysis calculation were added in Material and Methods Section (lines 231-250), according with USEPA Method. Default parameters used in the calculations of risk analysis are now detailed in Table 2. Further argumentation about your comment are present in Materials & Methods – Section 2.3.

About the comparisons of risks of both children and adults we highlighted this aspect at lines 312-317 and in the abstract at lies 37-38; we also add this consideration at lines 368-372.

Further comments:

INTRODUCTION

- *Line 117-120: Information concerning EU legislation on carcinogenic metals (As, Cd, Ni) in ambient air should be completed: indicated targets are annual ones and determined in PM10.*

  Done, as you required. The added part is highlighted at lines 128-130.

- *Line 121-132: rephrase this section and clearly state the objectives of this work. Why information about "historical evolution of size fractioned PM10 evolution of the ". Is it relevant to this work? Please clarify/rephrase.*

  Done. In particular a repeated statement was deleted. As yet affirmed in the manuscript ,the historical evaluation based on a previous study on the same area (Casazza 2013) is fundamental since the change in the emission sources examined in that occasion is related to a different deposition of metals (and other pollutants not here investigated) on different fractions of PM10 (lines 138-143).

- *Line 128-131: Information about importance of risk assessment should be placed in the previously in introduction and not in the objectives. Please correct references within whole manuscript once space appears before comma ("al. ,").*

  Yes, we have added a brief comment about it, and is highlighted at lines 133-137. Also the references has been revised, taking into account your observation.

MATERIALS AND METHODS

Section 2.1:
Sampling site and its characteristic should be better described: define site (urban, background) and it major influences (traffic, industrial, etc.). What were the surroundings of sampling site?

Ok, sampling site and its characteristic are highlighted at lines 162-164.

- *Section 2.2:*
Information on quality control/assurance of analytical process should be introduced. LODs/LOQs are missing too.

Thank you, now details concerning quality control and LOD/LOQ were added and highlighted in the Materials and Methods Section, as you required (lines 188-191).

- *Section 2.3:*
1. The non-carcinogenic and carcinogenic risks to particulate trace elements were calculated according to the USEPA methodology. Information concerning USEPA risk guidelines should be therefore included (THQ less than 1; for CR considers risk range 10-6 to 10-4: values below 10-6 for individual chemicals and pathways will cause negligible cancer risks but caution is recommended to ensure that the cumulative cancer risk for all potential carcinogenic contaminants does not have a residual cancer risk exceeding 10 −4).

Information concerning non-carcinogenic and carcinogenic risks guidelines are highlighted at lines 210-214 and lines 221-229 respectively.

- *2. Authors should justify the selected values for the following parameters in Table 1: BW, IR, Bo. For example inhalation rate of 0.9 L/min correspond to what level of physical activity? Please see USEPA materials concerning the respective information for adult and children, respectively:*
USEPA Exposure Factors Handbook: http://www.epa.gov/ncea/efh/pdfs/efh-complete.pdf Child-Specific Exposure Factors Handbook: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=199243
In addition, EFg of 6h/day (25%) for outdoor exposure seems rather high once people spend indoors typically 80-95%. Once again authors should justify/correct the selected value.

Thank you for your observation. Simplified information were embedded in the caption of Table 1, now named Table 2; the table now receive the English acronyms of singular parameters, as well as the correct references (USEPA 1991, USEPA 2009 - Part F and USEPA 2011). The inhalation rates (IR) were 0,9 m^3/h for adult and 0,7 m^3/h for children considering male and female combined for sedentary and light activity (USEPA 2011). From "Standard Default Exposure Factors" (USEPA 1991) and from USEPA 2009 Part F, Exposure Frequency (EF), Exposure Duration (ED) and Body Weight (BW) parameters were inferred, taking into account the "Inhalation of contaminant" as exposure pathway in a "residential scenario". An example of a residential scenario could consist of inhalation exposure for up to 24 hours per day, up to 350 days per year for 6 to 24 years for child and adult respectively (USEPA 2009 - Part F).

The methodology procedure for the estimation of inhalation exposure was detailed in USEPA 2009 – Part F Document.
Concerning the exposure parameters related to EFg value (now daily Exposure Time= ET), the USEPA 1991 Document indicates "24 hours, the whole day" as "resident air exposure time" and then setting in according with time spent in a specific site. Actually, in the light of your suggestions, an ED value of 6

hours seems rather high, thus as recommended by USEPA table of time spent outdoor (mean of 289 min/day for maximum age range considered) and according with lower limit indicated by you (80-95% time spent indoor), we have correct it considering 5 hours as ED value. Calculation were again performed.

- *3. Finally, authors estimate risks due to exposure to metals in PM10 in ambient air. Assumingly these are inhalation risks but the THQ and CR calculation were done using reference dose (RfD) and cancer slope (CSF) for ingestion exposure. It is not clear to me why authors did not use the USEPA methodology for inhalation exposure (Chronic Inhalation Reference Concentration RfC (mg/m3); Chronic Inhalation Unit Risk IUR (µg/m3)-1 (THQ, TR). The available information can be found here: (section 4.9.1-2): http://www.epa.gov/reg3hwmd/risk/human/rb-concentration_table/usersguide.htm*
  I think that their approach needs major revisions.

  Thank you for the observation. As told before (your comment n°2) , it was a spelling mistake.
  In the light of your suggestions, we have checked the USEPA methodology and new calculations were performed, taking into account the most recent USEPA update (November 2013) concerning RfC and IUR values, as added in the manuscript (page 9, lines 231-242).

- *4. Table 2: Reference [17]? Missing in the list of references (ISS-ISPESL).It is difficult to find/confirm data in Italian database. Furthermore, once USEPA approach is used, RfD and CSF referred by USEPA should be use: please consult the following link for Regional Screening Level (RSL) Summary Table: http://www.epa.gov/reg3hwmd/risk/human/rb-concentration_table/Generic_Tables/docs/master_sl_table_run_NOV2013.pdf*

  "Table 2" is now entitled "Table 1"; here the updated values from USEPA 2013 updated Table were reported as you suggested, and new specifications about it were provided in the text in the Material and Methods Section.

RESULT

- *Lines 222-233 (section 3.1) should be enhanced: authors compared average metals concentrations of 3 days measurements with annual targets. In addition, according to the statement in lines 140-142 the measurements were performed during winter season with greater exposure i.e. metals levels in ambient air; the EU target values might not be exceeded during other seasons.*

  Thank you, we added a sentence at lines 276-277 to enforce your observation.

- *Lines 264-265 This statement is obvious once PM10 contains both fractions. Remove/change.*
  Lines 265-266: Considering different deposition places of both fractions the statement "indeed total PM10……" is not correct. I am not sure what authors want to state. Furthermore according to Figure 1 most of the some metals (are predominantly (up to 70%) found in fine fraction.

  Ok, we removed the sentence about the PM10 inhalation and we improved the text (lines 300-303).

DISCUSSION

- *Line 288-300 this section should be placed in introduction*

  Ok, we moved the sentence in the introduction (lines 111-117).

- *Line 301: Mn causes carcinogenic risks…. CR risk assessment of Mn was not performed.*
  Authors should add discussion on different species metals and potential impacts. Finally, I believe that changes in calculations of THQ, CR using IUR, RfC will cause major changes/alterations which should be reflected in results/discussion.

  Thank you for the observation. It was a mistake we added toxic referred to Mn (lines 349-350). We also discussed about different species metals and potential impacts (lines 350-362).

PM10 size distribution of metals and environmental-sanitary risk analysis in the city of Torino

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ABSTRACT

The mechanisms responsible for negative biological effects due to airborne particulate matter (PM) exposure are still being studied, however the interactions between metals and biologic systems seem to be of primary importance. The aim of the study was to estimate a healthy risk linked to exposure to airborne PM10 metals by means of an environmental-sanitary risk assessment. Metals PM10 size distribution analysis was carried out in a central site of Torino city-Italy, then the Total Hazard Quotient (THQ) and the Cancer Risk (CR) were applied, according to standard EPA methods.

All sampled metals were present on the different PM10 fractions, however some metals were distributed in some specific fractions: ANOVA test shows Cr, Cu, Mo and Pb as differently distributed among the eight fractions, rising the hypothesis of potential effects in specific tracts of respiratory system. Regarding the risk assessment, in general the CR was higher for an adult than for a child, conversely the THQ resulted higher for a child.

While the concentrations of all the sampled metals appeared to be under control, their presence in the different PM10 fractions and their THQ and CR provided indications related to the body districts potentially in contact with these substances.

HIGHLIGHTS:

- Traces metals are differently distributed among the PM10 fractions
- The metals distribution in PM fractions can be useful for sanitary risk assessment
- Sanitary risk assessment shows potential risks for exposure to some metals on PM

KEY WORDS:

PM10, metals, health-sanitary risk analysis

CONFLICT OF INTEREST:

Authors declare no conflict of interest.

ACKNOWLEDGEMENTS:

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1. INTRODUCTION

Airborne particulate matter (PM) can be classified as PM10 (particles with an aerodynamic diameter Ø less than 10 μm), PM2.5 (Ø < 2.5 μm) and PM1 (Ø < 1 μm). The *coarse* fraction (PM10, 2.5–10 μm) has predominantly natural sources (geological material, such as fugitive and resuspended dust, and biological material, such as pollen and endotoxins), and its composition changes depending on the geology of the site considered. The *fine* fractions (PM2.5 and PM1) are dominated by combustion derived particles, consisting mainly of organic and inorganic elements adsorbed onto the surface of a carbonaceous core (Bruggemann et al., 2009) and secondary particles produced by photochemical reactions in the atmosphere (sulphates and nitrates). The carbonaceous fraction consists of aggregates of organic and inorganic carbon on which are adsorbed transition metals (Pb, Cd, V, Ni, Cu, Zn, Mn, Fe), organic compounds and biological constituents (USEPA, 1996). Coarse particles are mainly deposited in the extrathoracic region, while some inhaled fine particles reach the alveolar region of the lung (Kawanaka et al., 2011). The PM coarse fraction has been associated with pro-inflammatory and cytotoxic effects (Gualtieri et al., 2010; Hetland et al., 2004; Schiliro' et al., 2010); the PM fine fractions have been associated mainly to a higher genotoxic potential (Billet et al., 2008; Bonetta et al., 2009; de Kok et al., 2005; Traversi et al., 2009).

The mechanisms responsible for these biological effects have been continuously undergone review, and many questions still remain around some relevant aspects, for example specific dimensional fraction, number or mass of the particles, chemical components, among which metal compounds are relevant species. The metals and their compounds are found distributed among various dimensional fractions of the PM in the atmosphere. The transport and distribution of aerosol particles strictly depends on their size, besides on the weather conditions (Poschl, 2005; Stone et al., 2009): for this reason concentration, composition and size distribution of atmospheric metals particles are temporally and spatially highly variable. On the other hand the size particles depends mainly from emission sources: typically those emitted from anthropogenic sources are smaller than those emitted

from natural sources (Harrison et al., 2012). Metals are associated both to the coarse and to the fine fractions, in which they occur generally as different chemical compounds and in different oxidation state. On the basis of existing scientific evidence, many metals (often depending on their oxidation state) may have a direct or indirect active role in the mechanisms of PM biological action (Ziemacki et al., 2003).

Many metals are physiologically present in the human body as metal-enzymes or metal-protein such as iron (catalase and heme), zinc (RNA polymerase, carbonic anhydrase, Cu–Zn superoxide dismutase, angiotensin I converting enzyme), copper (superoxide dismutase, cytochrome oxidase, dopamine hydroxylase, and several other oxidases that reduce molecular oxygen) and manganese (mitocondrial Mn superoxide dismutase, glutamine synthetase, arginase, and activates several hydrolases, transferases and carboxylases) (Davis and Greger, 1992; Fraga, 2005; Hamilton et al., 2000; Kanumakala et al., 2002). Some chemical-physical factors such as hydrosolubility, dimensional distribution and the incorporation into aerosol particles, could influence the bioavailability of metals in PM. Since toxic and carcinogenic properties are well known for many metals, research on deposition of particle-bound mutagens in the atmosphere - first of all at respiratory level - is demanded for assessment of the influence of PM on human health. Thus deeper investigations on atmospheric concentrations, chemical characteristics and physical-chemical properties of the various metals present in the atmosphere are generally more limited respect to the past. This is also true for the potential risks to which humans are undergone in relation to their chronic presence in atmosphere. As metals represent hazard to human health, careful monitoring should be considered. Furthermore the investigation of the health risks associated with airborne metals may provide useful information regarding environmental risks of outdoor environments. The Directive 2008/50/EC of the European Parliament and of the Council establishes annual target values for the concentration of As (6 ng m^{-3}), Cd (5 ng m^{-3}) and Ni (20 ng m^{-3}) (determined on PM10 fraction) in ambient air so as to avoid, prevent or reduce harmful effects of these substances

on human health and the environment as a whole (Italian transposition of Directive: D.Lgs. 155, 2010).

In the international context, the procedure of environmental–sanitary risk assessment has assumed a central role in the management of contaminated environments, mainly with reference to emerging pollutants but the same procedure can be successfully applied to other fields of interest, such as surface waters, contaminated sites, foods and pharmaceuticals (Jin et al., 2012; Ruffino et al., 2013; Stasinakis et al., 2012).

In order to address these issues, after an historical data comparison considering three representative periods with changes of emission characteristic (Casazza et al., 2013), size distributions of trace metals of specific health concern in size fractionated PM10 were evaluated. Samples were collected in a urban site (traffic oriented in the urban area of Torino, a north Italian city). Finally, the environmental–sanitary risk assessment was carried out, in order to evaluate if the metals in the different PM fractions may pose a health risk to child or adult via inhalation of airborne PM.

2. MATERIALS AND METHODS

2.1 PM sampling

PM sampling was carried out in the city of Torino – Italy. The city has an area of 130,2 Km^2 and a population of 908.551 inhabitants. Our sampling occurred during three autumn/winter days in 2011. This choice is due to different factors. From one side, during this period the emission sources of atmospheric pollutants are more numerous and intense. In the same period some atmospheric phenomena occur, such as thermal inversions, that can lead to a greater exposure levels for the population (Cirera et al., 2009). The sampling days were chosen considering the presence of stable good weather conditions, in order to exclude any macroscopic effect due to transport and scavenging phenomena. The mean temperature and humidity during the sampling period were 15.5 °C and 71% respectively. PM sampling was performed using an Andersen 2000 Inc. 'Mark II' model

8-stages cascade impactor, which allows aerosol particles subdivision with respect to different cut-off diameters: 0.54, 0.88, 1.60, 2.70, 4.00, 5.85, 9.00 and 11 μm. The cascade impactor was connected to an Andersen 2000 Inc. model 22-000 pump, with a selected air flux of 28.3 l/min, checked through a volumetric measurer. For collecting the particle fractions, we used Whatman no. 5 cellulose acetate filters, having a diameter of 110 mm, that were weighted, before and after the field measures, using an analytical scale having a resolution of 0.001 mg. The sampling site was located outdoors, fifteen-m high, in central zone of the city, with residential/commercial emissions, ''sandwiched'' between two busy streets and not far from a green area. After weighing, the filters were stored in a freezer at -18 °C until the metal extraction process.

2.2 Filter extraction and metals quantification

Following the air sampling, different metals were quantified from each of the eight filters of the sampler; the quantified metals were: V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, Rh, Pd, Cd, Sn, Pt and Pb. In particular, after the acid attack in a microwave digestor (Milestone, MLS-1200 Mega) through a mixture 1:4 of hydrogen peroxide (30% Fluka pa) and Carlo Erba HNO3 65% purified by sub-boiling, the quantification has been made. double focusing magnetic sector inductively coupled plasma mass spectrometer (Thermo Finnigan Element 2). Mass resolution and isotope selection were optimized for each element to ensure resolution of spectral interferences and maximize sensitivity. A minimum of triplicate 180 s analyses on each sample was conducted following a 60 s uptake and stabilization period. Between samples the nebulizer system was rinsed for 2 min with 2% sub-boiled HNO_3, which eliminated carry-over and reconditioned the sampler cone. Sets of instrumental blank and calibration verification checks were run at frequent intervals during the batch sequence. The calibrations were performed with standard solutions prepared in aliquots of sample blanks. Process blanks were incorporated into the dissolution and analytical procedure to assess metal contribution from the filters, bombs, Milli-Q water and purified acids used in this

procedure. All signals for samples were obtained after subtraction of their appropriate process blank values. The relative standard deviation for all elements in each sample was always lower than 5 %. NIST SRM 1648a (Urban Particulate Matter) was used to verify that analyte concentrations were within 15% of the expected values before proceeding with sample analysis. The analytical process conditions were: (1) plasma power: 1270 W; (2) gas flux through the nebulizer: 1.07 L min^{-1}; (3) auxiliary gas flux: 1.1 L min^{-1}; (4) plasma gas flux: 15 L min^{-1}; (5) peristaltic pump speed: 7 rpm; (6) integration time: 10 s; (7) scanning: 9 (low resolution) and 12 (medium resolution). The LODs and LOQs in the conditions reported were, respectively, under 0.01 and 0.03 ng L^{-1} for all the element quantified, which resulted in minimum detectable and quantifiable amounts referred to the volume of air sampled (280 m^3) of 0.002 and 0.006 ng m^{-3}.

2.3 Risk analysis

A risk assessment was carried out. The receptors were adults and children living in Torino. The sources of contamination were the different PM10 fractions, in which chemicals were considered as stratified on the 3 main pooled PM10 fractions: coarse, PM2.5 and PM1. The chemicals of concern involved in the risk assessment were V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, Rh, Pd, Cd, Sn, Pt, Pb. Among the possible routes, in this work, only inhalation of dusts and gases from PM was taken into account, since it is the most important exposure route to PM of health concern. In risk quantification for each receptor (child or adult), the risks due to each contaminant were divided into two categories: non-carcinogens and carcinogens.

According to standard EPA methods (USEPA, 1989; USEPA, 2009; USEPA, 2011) for non-carcinogenic chemicals, the risk target hazard quotient (THQ) (dimensionless) was calculated as in the following equation:

$$\textbf{(1)} \qquad THQ = \frac{ADI}{RfDi}$$

where ADI, average daily intake (mg/kg/day), is the estimated dose the receptor is exposed to from an exposure route; RfD*i*, reference concentration (mg/kg day), is the dose, for a given route, that is believed to be without effect; the cumulative THQ has to be seen as the sum of the THQ calculated as in Eq. (1) for each contaminant. THQ assumes that there is a level of exposure (i.e., RfD*i*) below which it is unlikely for even sensitive populations to experience adverse health effects. If the exposure level (ADI) exceeds unity, there may be concern for potential noncancer effects; higher values of THQ (above unity) indicate the greater levels of concern (USEPA, 2011).

For carcinogenic chemicals, the cancer risk (CR) (dimensionless) was calculated as in the following equation:

$$\mathbf{(2)} \qquad CR = LDI \times CSF$$

where CR is the probability of cancer occurring in the exposed population over a 70-year lifetime; LDI, lifetime daily intake (mg/kg/day), is the dose of contaminants the receptor is exposed to for all their life through an exposure route; CSF (mg kg^{-1} $day^{-1})^{-1}$ is the cancer slope factor for each exposure route derived from dose–response studies. The carcinogenic risks were assessed as the incremental probability of an individual to develop cancer, over a lifetime, as a result of exposure to that potential carcinogen (i.e., incremental or excess individual lifetime cancer risk). CR considers risk range 10^{-4} (risk of developing cancer over a human lifetime is 1 in 10.000) to 10^{-6} (risk of developing cancer over a human lifetime is 1 in 1.000.000): values below 10^{-6} for individual chemicals and pathways will cause negligible cancer risks but caution is recommended to ensure that the cumulative cancer risk for all potential carcinogenic contaminants does not have a cancer risk exceeding 10^{-4} (USEPA, 2011). However cumulative CR the maximum acceptable value is 10^{-5}. With reference to one route of exposure, the cumulative CR is the sum of the CR calculated as in Eq. (2) for each contaminant.

Both the RfD*i* and CSF values were derived from existent reference concentrations (RfC) and from Inhalation Unit Risk (IUR) respectively – if available (USEPA, 2013a), according to the USEPA derivation (USEPA, 2013b):

$$\textbf{(3)} \qquad RfDi = \frac{(RfC \times IR \times AR)}{(BW \times 100)}$$

$$\textbf{(4)} \qquad CSF = IUR \times (BW/IR) \times 1000$$

where RfC is the USEPA reference concentration – if available (mg m^{-3}), IUR is the Inhalation Unit Risk (µg $m^{-3})^{-1}$, IR and BW are the inhalation rate and body weight of an adult (20 m^3 day^{-1} and 70 kg, and AR is the absorption rate (100%) **(Table 1)**.

The daily intake, ADI for non-carcinogenics and LDI for carcinogenics, is the product of the specific exposition rate (E, daily amount, normalized on the body weight, of crumb rubber or rainwater contacted, or air breathed) and the concentration C (ADI and LDI = E x C).

The exposition rate was calculated in the following equations:

$$\textbf{(5)} \quad E\ (mg/kg\ day) = \frac{(IR \times ET \times EF \times ED)}{(BW \times AT)}$$

The parameters utilized to calculate the specific exposition rates are listed in **Table 2**. For the non-carcinogenic ADI values, the average time was assumed to be equal to 6 years for children and 24 years for adults (equal to ED), while 70 years (lifetime) was assumed for the calculation of LDI for carcinogenic substances.

In the Risk analysis the C of metals on PM was considered to be equal to the concentration at the point of exposure (although this assumption is clearly not correct) and the parameter utilized for the calculation of the specific exposition rates were highly conservative.

2.4 Statistical analysis

One-way analysis of variance (ANOVA) were applied to evaluate any differences in the distribution of metals among both the eight singular samplers stages (0.54, 0.88, 1.60, 2.70, 4.00, 5.85, 9.00 and 11 µm) and the three aggregated samplers stages (coarse, PM2.5 and PM1). With this purpose the homogeneity of the variance was firstly assessed through the Levene test, thus the equal variance of

Tukey's test was assumed for post hoc multiple comparisons. Finally, a P value of ≤ 0.05 (two-tailed) was considered to be significant for all tests. All of the statistical analyses were performed using SPSS Package, version 19.0.

3. RESULTS

3.1 Air sampling and size-fractionated distribution of airborne metals

The mean of PM10 and PM2.5 were 98 ± 1 μg m^{-3} and 83 ± 1 μg m^{-3} respectively. These values were high and about twice taking into account the quality target (European Directive 2008/50/EC) of a daily value for PM10 of 50 μg m^{-3}. The PM2.5/PM10 ratio ranged from 75% to 81%. Referring to the annual limits of metals set by the European Directive 2008/50/EC, these are observed excluding for Cd for which our measurements exceed the target value of 5 ng m^{-3}, being more than one order of magnitude higher than the limit (**Table 3**), even if our sampling reflects only a "spot" situation (daily) and the European limits refer to the yearly average. With reference to environmental monitoring at local and national level by ARPA - Piedmont competence (Agenzia Regionale per la Protezione dell'Ambiente – Piemonte), 2011 was the warmest year observed in Piedmont in the last 50 years, and concerning the precipitations an abnormal lack of rains occurred during the generally wettest months (including October). This is in addition to the conditions of atmospheric instability particularly intense in winter months, resulted in an accumulation of pollutants, especially powders and other chemical compounds, including metals. Thereafter it should be noted that in other years or seasons the EU target values might not be exceeded. The metals concentrations in the airborne particulate were below the limits set by the European Directive 2008/50/EC, due to the significant reduced presence on the surrounding territory of mining and metallurgy, as well as the ongoing disposal of large coal-fired power plants and fuel oil, which are the main anthropogenic sources of heavy metals, such as As and Pb.

289 Although the global situation of the air seems to be adequately under control, a different meaning to
290 the fractionate-size sampler is given. In fact, the health significance associated with an exposure
291 through the component fractions forming the PM10 (*fine* and *coarse* fractions), determined the need
292 to measure the presence of metals in these fractions particle size collected at the same time, but
293 separately, since they have different capacity of deposition in the various regions of the respiratory
294 tract, expressing a different and specific biological action.
295 All sampled metals are present and variously distributed on all the eight sampler stages, however
296 some metals are stratified in specific sampler stages: ANOVA test shows Cr, Cu, Mo and Pb as
297 statistically and differently distributed among the eight sampler stages; in particular Cr is
298 preferentially accumulated on the finer filter 0.54 µm, Cu and Mo are localized on 2.70 µm filter,
299 and finally Pb is accumulated on the 11 µm filter (**Table 3).** Metals contributions were then merge
300 in accordance with the dimensional classification of *coarse*, PM2.5 and PM1 **(Figure 1)**. In this
301 case the ANOVA test confirmed a statistically significant dimensional-dependent distribution of the
302 same metals previously identified, also adding Ni, As and Pt; in particular As, Ni, Mo and Pb are
303 preferentially accumulated on the *coarse* fraction, Cu on PM 2.5 and finally Pt and Cr on PM1.
304 Published information about the size distribution of metals in urban atmosphere are not so extended;
305 although relatively high proportions of pollutants including transition metals are regularly found in
306 ultrafine PM (Sioutas et al., 2005). Considering their small size, ultrafine particles (and all that can
307 be conveyed by them) can also readily traverse biological membranes, facilitating systemic
308 distribution in the body and eventually revealing multiple local or systemic effects.
309
310 *3.2 Risk analysis*
311 The cumulative risk values from non-carcinogenic substances, THQ, calculated as in Eq. (1) for
312 each metals, for each PM principal fraction and for each receptors (adult and child) are shown in
313 **Figures 2**; these values ranged from 4.96×10^{-4} and 1.69 for adult while from 1.80×10^{-3} and 6.13 for
314 child. The cumulative risk values from carcinogenic substances, CR, calculated as in Eq. (2), for

each metals, for each PM principal fraction and for each receptors (adult and child) are shown in **Figures 3**; these values ranged from $3.71x10^{-8}$ and $1.04x10^{-5}$ for adult while from $5.84x10^{-8}$ and $9.46x10^{-6}$ for child. The non-carcinogenic risk was higher for children than for adults, in line with the fact that children are more sensitive to non-carcinogenic substances than adults **(Figure 2)** and the CR were comparable – in terms of order of magnitude – for adult and child. The highest THQ values for both adult and child were reported for Mn and Cd, in particular the *coarse* fraction was the more dangerous for Mn and the PM2.5 fraction for Cd.

Regarding the CR the highest value for both adult and child was reported for Cd without significant differences in PM fractions; the *coarse* fraction was the more dangerous for Co and As.

Given the observed differences it is fundamental to consider that any changes in the fractions amount in PM10 may correspond to a different metals intake and a different risk (carcinogenic and non-carcinogenic).

The results of the risk analysis showed that for the examined metals, the CR proved to be lower than 10^{-6} and the non-carcinogenic risk, THQ, lower than 1, in line with European guidelines with the exception of Cd, for which the total PM10 THQ were 1.69 and 6.13 while total PM10 CR were $1.04x10^{-5}$ and $9.46x10^{-6}$ for adult and child respectively), this result was clearly correlated to the high and remarkable Cd concentrations evaluated in this study probably due to the working activities related to the restoring of a building nearby the sampling site. We must also account the fact that exposure to metals occurs also via ingestion and dermal contact and, if these routes are considered, the estimated risks might be higher (Slezakova et al., 2013).

4. DISCUSSION

The composition of PM is very variable and depends on many different factors among which sources, climate and topography are only few examples. The chemical speciation of PM has been under study for many decades and the presence of heavy metals is known from past and recent literature (Chiari et al., 2006; Pey et al., 2010). Nevertheless there are few works that associate the

PM metals distribution to health effects stratified by dimensional particles size (Slezakova et al., 2013) by means of environmental-sanitary risk assessment. By our samplings metals appear to be variously distributed among all the fractions demonstrating their background presence. While the concentrations of all the sampled metals appear to be under control, consideration should be given to the significantly different presence of certain metals in the different PM fractions, which provides an indication related to the metals ability to penetrate into the respiratory tracts. Metals found in the finer fractions are potentially able to deeply penetrate into the bloodstream through the alveoli, to be carried far from respiratory tract.

In particular our results highlight Mn and Cd as the two metals reflecting the potential toxic or carcinogenic risks for human health. Mn is reported to be essential for the development of the brain, being the concentrations in human brain higher in adults (approximately 0.25 mg g^{-1} wet weight) than in infants, suggesting this metal as required for brain functions (Markesbery et al., 1984; Pomier-Layrargues et al., 1995; Takeda, 2003).When this metal is abnormally concentrated in the brain, especially in the basal ganglia, this results in neurological disorders similar to Parkinson's disease (Ono et al., 1995; Takeda, 2003), and neurotoxicity. Concerning Cd, due to its extremely protracted biological half-life (approximately 20–30 years in humans), low rate of excretion from the body and storage predominantly in soft tissues (primarily, liver and kidneys), this metal has a diversity of toxic effects including nephrotoxicity, carcinogenicity, teratogenicity and endocrine and reproductive toxicities. Current evidence suggests that exposure to Cd induces genomic instability through complex and multifactorial mechanisms. Most important seems to be Cd interaction with DNA mismatch repair mechanism (MMR) (Giaginis et al., 2006), and induction of apoptosis (Zarros, 2008).

Even if the levels of inhalation exposure to such metals associated with the PM may be considered too low to induce phenomena of toxicity according to the classical mechanisms, their presence - even in traces - could play an important role, being a chronic exposure factor, in the development of

biological mechanisms responsible for some of the recorded health effects on the local population, as confirmed by risk calculation.

In conclusion for all the examined metals, carcinogenic and non-carcinogenic, and for both children and adults, the inhalation of airborne PM10 in a urban site gave risk values about one order of magnitude lower than those indicated in guidelines with the exception of Cd (which reflects a "spot" situation); in this view the hazard quotient resulted higher for a child than for an adult while carcinogenic risk was similar. The absence of PM-metals risk in a urban site with high pollution levels (WHO, 2011) is concordant with the good reduction trend of metals in Europe (EEA, 2013) that however is a matter of concern for all the policies aimed at the protection of human health.

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FIGURES CAPTIONS

Figure 1: *Metals contribution (%) among the 3 main fractions of PM. Asterisks (*) provide an indication of statistically significant distribution (p<0.01, ANOVA test).*

Figure 2: *Cumulative risk values from non-carcinogenic substances – THQ. A) shows values for adults, B) shows values for children.THQ values are expressed as log-scale.*

Figure 3: *Cumulative risk values from carcinogenic substances – CR. A) shows values for adults, B) shows values for children. CR values are expressed as log-scale.*

PM10 size distribution of metals and environmental-sanitary risk analysis in the city of Torino

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ABSTRACT

The mechanisms responsible for negative biological effects due to airborne particulate matter (PM) exposure are still being studied, however the interactions between metals and biologic systems seem to be of primary importance. The aim of the study was to estimate a healthy risk linked to exposure to airborne PM10 metals by means of an environmental-sanitary risk assessment. Metals PM10 size distribution analysis was carried out in a central site of Torino city-Italy, then the Total Hazard Quotient (THQ) and the Cancer Risk (CR) were applied, according to standard EPA methods.

All sampled metals were present on the different PM10 fractions, however some metals were distributed in some specific fractions: ANOVA test shows Cr, Cu, Mo and Pb as differently distributed among the eight fractions, rising the hypothesis of potential effects in specific tracts of respiratory system. Regarding the risk assessment, in general the CR was higher for an adult than for a child, conversely the THQ resulted higher for a child.

While the concentrations of all the sampled metals appeared to be under control, their presence in the different PM10 fractions and their THQ and CR provided indications related to the body districts potentially in contact with these substances.

HIGHLIGHTS:

- Traces metals are differently distributed among the PM10 fractions
- The metals distribution in PM fractions can be useful for sanitary risk assessment
- Sanitary risk assessment shows potential risks for exposure to some metals on PM

KEY WORDS:

PM10, metals, health-sanitary risk analysis

CONFLICT OF INTEREST:

Authors declare no conflict of interest.

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1. INTRODUCTION

Airborne particulate matter (PM) can be classified as PM10 (particles with an aerodynamic diameter Ø less than 10 μm), PM2.5 (Ø < 2.5 μm) and PM1 (Ø < 1 μm). The *coarse* fraction (PM10, 2.5–10 μm) has predominantly natural sources (geological material, such as fugitive and resuspended dust, and biological material, such as pollen and endotoxins), and its composition changes depending on the geology of the site considered. The *fine* fractions (PM2.5 and PM1) are dominated by combustion derived particles, consisting mainly of organic and inorganic elements adsorbed onto the surface of a carbonaceous core (Bruggemann et al., 2009) and secondary particles produced by photochemical reactions in the atmosphere (sulphates and nitrates). The carbonaceous fraction consists of aggregates of organic and inorganic carbon on which are adsorbed transition metals (Pb, Cd, V, Ni, Cu, Zn, Mn, Fe), organic compounds and biological constituents (USEPA, 1996). Coarse particles are mainly deposited in the extrathoracic region, while some inhaled fine particles reach the alveolar region of the lung (Kawanaka et al., 2011). The PM coarse fraction has been associated with pro-inflammatory and cytotoxic effects (Gualtieri et al., 2010; Hetland et al., 2004; Schiliro' et al., 2010); the PM fine fractions have been associated mainly to a higher genotoxic potential (Billet et al., 2008; Bonetta et al., 2009; de Kok et al., 2005; Traversi et al., 2009).

The mechanisms responsible for these biological effects have been continuously undergone review, and many questions still remain around some relevant aspects, for example specific dimensional fraction, number or mass of the particles, chemical components, among which metal compounds are relevant species. The metals and their compounds are found distributed among various dimensional fractions of the PM in the atmosphere. The transport and distribution of aerosol particles strictly depends on their size, besides on the weather conditions (Poschl, 2005; Stone et al., 2009): for this reason concentration, composition and size distribution of atmospheric metals particles are temporally and spatially highly variable. On the other hand the size particles depends mainly from emission sources: typically those emitted from anthropogenic sources are smaller than those emitted

from natural sources (Harrison et al., 2012). Metals are associated both to the coarse and to the fine fractions, in which they occur generally as different chemical compounds and in different oxidation state. On the basis of existing scientific evidence, many metals (often depending on their oxidation state) may have a direct or indirect active role in the mechanisms of PM biological action (Ziemacki et al., 2003).

Many metals are physiologically present in the human body as metal-enzymes or metal-protein such as iron (catalase and heme), zinc (RNA polymerase, carbonic anhydrase, Cu–Zn superoxide dismutase, angiotensin I converting enzyme), copper (superoxide dismutase, cytochrome oxidase, dopamine hydroxylase, and several other oxidases that reduce molecular oxygen) and manganese (mitocondrial Mn superoxide dismutase, glutamine synthetase, arginase, and activates several hydrolases, transferases and carboxylases) (Davis and Greger, 1992; Fraga, 2005; Hamilton et al., 2000; Kanumakala et al., 2002). Some chemical-physical factors such as hydrosolubility, dimensional distribution and the incorporation into aerosol particles, could influence the bioavailability of metals in PM. Since toxic and carcinogenic properties are well known for many metals, research on deposition of particle-bound mutagens in the atmosphere - first of all at respiratory level - is demanded for assessment of the influence of PM on human health. Thus deeper investigations on atmospheric concentrations, chemical characteristics and physical-chemical properties of the various metals present in the atmosphere are generally more limited respect to the past. This is also true for the potential risks to which humans are undergone in relation to their chronic presence in atmosphere. As metals represent hazard to human health, careful monitoring should be considered. Furthermore the investigation of the health risks associated with airborne metals may provide useful information regarding environmental risks of outdoor environments. The Directive 2008/50/EC of the European Parliament and of the Council establishes annual target values for the concentration of As (6 ng m^{-3}), Cd (5 ng m^{-3}) and Ni (20 ng m^{-3}) (determined on PM10 fraction) in ambient air so as to avoid, prevent or reduce harmful effects of these substances

on human health and the environment as a whole (Italian transposition of Directive: D.Lgs. 155, 2010).

In the international context, the procedure of environmental–sanitary risk assessment has assumed a central role in the management of contaminated environments, mainly with reference to emerging pollutants but the same procedure can be successfully applied to other fields of interest, such as surface waters, contaminated sites, foods and pharmaceuticals (Jin et al., 2012; Ruffino et al., 2013; Stasinakis et al., 2012).

In order to address these issues, after an historical data comparison considering three representative periods with changes of emission characteristic (Casazza et al., 2013), size distributions of trace metals of specific health concern in size fractionated PM10 were evaluated. Samples were collected in a urban site (traffic oriented in the urban area of Torino, a north Italian city). Finally, the environmental–sanitary risk assessment was carried out, in order to evaluate if the metals in the different PM fractions may pose a health risk to child or adult via inhalation of airborne PM.

2. MATERIALS AND METHODS

2.1 PM sampling

PM sampling was carried out in the city of Torino – Italy. The city has an area of 130,2 Km^2 and a population of 908.551 inhabitants. Our sampling occurred during three autumn/winter days in 2011. This choice is due to different factors. From one side, during this period the emission sources of atmospheric pollutants are more numerous and intense. In the same period some atmospheric phenomena occur, such as thermal inversions, that can lead to a greater exposure levels for the population (Cirera et al., 2009). The sampling days were chosen considering the presence of stable good weather conditions, in order to exclude any macroscopic effect due to transport and scavenging phenomena. The mean temperature and humidity during the sampling period were 15.5 °C and 71% respectively. PM sampling was performed using an Andersen 2000 Inc. 'Mark II' model

8-stages cascade impactor, which allows aerosol particles subdivision with respect to different cut-off diameters: 0.54, 0.88, 1.60, 2.70, 4.00, 5.85, 9.00 and 11 μm. The cascade impactor was connected to an Andersen 2000 Inc. model 22-000 pump, with a selected air flux of 28.3 l/min, checked through a volumetric measurer. For collecting the particle fractions, we used Whatman no. 5 cellulose acetate filters, having a diameter of 110 mm, that were weighted, before and after the field measures, using an analytical scale having a resolution of 0.001 mg. The sampling site was located outdoors, fifteen-m high, in central zone of the city, with residential/commercial emissions, ‘‘sandwiched’’ between two busy streets and not far from a green area. After weighing, the filters were stored in a freezer at -18 °C until the metal extraction process.

2.2 Filter extraction and metals quantification

Following the air sampling, different metals were quantified from each of the eight filters of the sampler; the quantified metals were: V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, Rh, Pd, Cd, Sn, Pt and Pb. In particular, after the acid attack in a microwave digestor (Milestone, MLS-1200 Mega) through a mixture 1:4 of hydrogen peroxide (30% Fluka pa) and Carlo Erba HNO3 65% purified by sub-boiling, the quantification has been made. double focusing magnetic sector inductively coupled plasma mass spectrometer (Thermo Finnigan Element 2). Mass resolution and isotope selection were optimized for each element to ensure resolution of spectral interferences and maximize sensitivity. A minimum of triplicate 180 s analyses on each sample was conducted following a 60 s uptake and stabilization period. Between samples the nebulizer system was rinsed for 2 min with 2% sub-boiled HNO_3, which eliminated carry-over and reconditioned the sampler cone. Sets of instrumental blank and calibration verification checks were run at frequent intervals during the batch sequence. The calibrations were performed with standard solutions prepared in aliquots of sample blanks. Process blanks were incorporated into the dissolution and analytical procedure to assess metal contribution from the filters, bombs, Milli-Q water and purified acids used in this

procedure. All signals for samples were obtained after subtraction of their appropriate process blank values. The relative standard deviation for all elements in each sample was always lower than 5 %. NIST SRM 1648a (Urban Particulate Matter) was used to verify that analyte concentrations were within 15% of the expected values before proceeding with sample analysis. The analytical process conditions were: (1) plasma power: 1270 W; (2) gas flux through the nebulizer: 1.07 L min^{-1}; (3) auxiliary gas flux: 1.1 L min^{-1}; (4) plasma gas flux: 15 L min^{-1}; (5) peristaltic pump speed: 7 rpm; (6) integration time: 10 s; (7) scanning: 9 (low resolution) and 12 (medium resolution). The LODs and LOQs in the conditions reported were, respectively, under 0.01 and 0.03 ng L^{-1} for all the element quantified, which resulted in minimum detectable and quantifiable amounts referred to the volume of air sampled (280 m^3) of 0.002 and 0.006 ng m^{-3}.

2.3 Risk analysis

A risk assessment was carried out. The receptors were adults and children living in Torino. The sources of contamination were the different PM10 fractions, in which chemicals were considered as stratified on the 3 main pooled PM10 fractions: coarse, PM2.5 and PM1. The chemicals of concern involved in the risk assessment were V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, Rh, Pd, Cd, Sn, Pt, Pb. Among the possible routes, in this work, only inhalation of dusts and gases from PM was taken into account, since it is the most important exposure route to PM of health concern. In risk quantification for each receptor (child or adult), the risks due to each contaminant were divided into two categories: non-carcinogens and carcinogens.

According to standard EPA methods (USEPA, 1989; USEPA, 2009; USEPA, 2011) for non-carcinogenic chemicals, the risk target hazard quotient (THQ) (dimensionless) was calculated as in the following equation:

$$\textbf{(1)} \qquad THQ = \frac{ADI}{RfD_i}$$

where ADI, average daily intake (mg/kg/day), is the estimated dose the receptor is exposed to from an exposure route; RfD*i*, reference concentration (mg/kg day), is the dose, for a given route, that is believed to be without effect; the cumulative THQ has to be seen as the sum of the THQ calculated as in Eq. (1) for each contaminant. THQ assumes that there is a level of exposure (i.e., RfD*i*) below which it is unlikely for even sensitive populations to experience adverse health effects. If the exposure level (ADI) exceeds unity, there may be concern for potential noncancer effects; higher values of THQ (above unity) indicate the greater levels of concern (USEPA, 2011).

For carcinogenic chemicals, the cancer risk (CR) (dimensionless) was calculated as in the following equation:

$$\mathbf{(2)} \qquad CR = LDI \times CSF$$

where CR is the probability of cancer occurring in the exposed population over a 70-year lifetime; LDI, lifetime daily intake (mg/kg/day), is the dose of contaminants the receptor is exposed to for all their life through an exposure route; CSF (mg kg^{-1} $day^{-1})^{-1}$ is the cancer slope factor for each exposure route derived from dose–response studies. The carcinogenic risks were assessed as the incremental probability of an individual to develop cancer, over a lifetime, as a result of exposure to that potential carcinogen (i.e., incremental or excess individual lifetime cancer risk). CR considers risk range 10^{-4} (risk of developing cancer over a human lifetime is 1 in 10.000) to 10^{-6} (risk of developing cancer over a human lifetime is 1 in 1.000.000): values below 10^{-6} for individual chemicals and pathways will cause negligible cancer risks but caution is recommended to ensure that the cumulative cancer risk for all potential carcinogenic contaminants does not have a cancer risk exceeding 10^{-4} (USEPA, 2011). However cumulative CR the maximum acceptable value is 10^{-5}. With reference to one route of exposure, the cumulative CR is the sum of the CR calculated as in Eq. (2) for each contaminant.

Both the RfD*i* and CSF values were derived from existent reference concentrations (RfC) and from Inhalation Unit Risk (IUR) respectively – if available (USEPA, 2013a), according to the USEPA derivation (USEPA, 2013b):

$$\textbf{(3)} \qquad RfDi = \frac{(RfC \times IR \times AR)}{(BW \times 100)}$$

$$\textbf{(4)} \qquad CSF = IUR \times (BW/IR) \times 1000$$

where RfC is the USEPA reference concentration – if available (mg m^{-3}), IUR is the Inhalation Unit Risk ($\mu g\ m^{-3})^{-1}$, IR and BW are the inhalation rate and body weight of an adult (20 $m^3\ day^{-1}$ and 70 kg, and AR is the absorption rate (100%) **(Table 1)**.

The daily intake, ADI for non-carcinogenics and LDI for carcinogenics, is the product of the specific exposition rate (E, daily amount, normalized on the body weight, of crumb rubber or rainwater contacted, or air breathed) and the concentration C (ADI and LDI = E x C).

The exposition rate was calculated in the following equations:

$$\textbf{(5)}\ E\ (mg/kg\ day) = \frac{(IR \times ET \times EF \times ED)}{(BW \times AT)}$$

The parameters utilized to calculate the specific exposition rates are listed in **Table 2**. For the non-carcinogenic ADI values, the average time was assumed to be equal to 6 years for children and 24 years for adults (equal to ED), while 70 years (lifetime) was assumed for the calculation of LDI for carcinogenic substances.

In the Risk analysis the C of metals on PM was considered to be equal to the concentration at the point of exposure (although this assumption is clearly not correct) and the parameter utilized for the calculation of the specific exposition rates were highly conservative.

2.4 Statistical analysis

One-way analysis of variance (ANOVA) were applied to evaluate any differences in the distribution of metals among both the eight singular samplers stages (0.54, 0.88, 1.60, 2.70, 4.00, 5.85, 9.00 and 11 μm) and the three aggregated samplers stages (coarse, PM2.5 and PM1). With this purpose the homogeneity of the variance was firstly assessed through the Levene test, thus the equal variance of

Tukey's test was assumed for post hoc multiple comparisons. Finally, a P value of ≤ 0.05 (two-tailed) was considered to be significant for all tests. All of the statistical analyses were performed using SPSS Package, version 19.0.

3. RESULTS

3.1 Air sampling and size-fractionated distribution of airborne metals

The mean of PM10 and PM2.5 were 98 ± 1 μg m^{-3} and 83 ± 1 μg m^{-3} respectively. These values were high and about twice taking into account the quality target (European Directive 2008/50/EC) of a daily value for PM10 of 50 μg m^{-3}. The PM2.5/PM10 ratio ranged from 75% to 81%. Referring to the annual limits of metals set by the European Directive 2008/50/EC, these are observed excluding for Cd for which our measurements exceed the target value of 5 ng m^{-3}, being more than one order of magnitude higher than the limit (**Table 3**), even if our sampling reflects only a "spot" situation (daily) and the European limits refer to the yearly average. With reference to environmental monitoring at local and national level by ARPA - Piedmont competence (Agenzia Regionale per la Protezione dell'Ambiente – Piemonte), 2011 was the warmest year observed in Piedmont in the last 50 years, and concerning the precipitations an abnormal lack of rains occurred during the generally wettest months (including October). This is in addition to the conditions of atmospheric instability particularly intense in winter months, resulted in an accumulation of pollutants, especially powders and other chemical compounds, including metals. Thereafter it should be noted that in other years or seasons the EU target values might not be exceeded. The metals concentrations in the airborne particulate were below the limits set by the European Directive 2008/50/EC, due to the significant reduced presence on the surrounding territory of mining and metallurgy, as well as the ongoing disposal of large coal-fired power plants and fuel oil, which are the main anthropogenic sources of heavy metals, such as As and Pb.

289 Although the global situation of the air seems to be adequately under control, a different meaning to
290 the fractionate-size sampler is given. In fact, the health significance associated with an exposure
291 through the component fractions forming the PM10 (*fine* and *coarse* fractions), determined the need
292 to measure the presence of metals in these fractions particle size collected at the same time, but
293 separately, since they have different capacity of deposition in the various regions of the respiratory
294 tract, expressing a different and specific biological action.
295 All sampled metals are present and variously distributed on all the eight sampler stages, however
296 some metals are stratified in specific sampler stages: ANOVA test shows Cr, Cu, Mo and Pb as
297 statistically and differently distributed among the eight sampler stages; in particular Cr is
298 preferentially accumulated on the finer filter 0.54 µm, Cu and Mo are localized on 2.70 µm filter,
299 and finally Pb is accumulated on the 11 µm filter (**Table 3).** Metals contributions were then merge
300 in accordance with the dimensional classification of *coarse*, PM2.5 and PM1 **(Figure 1)**. In this
301 case the ANOVA test confirmed a statistically significant dimensional-dependent distribution of the
302 same metals previously identified, also adding Ni, As and Pt; in particular As, Ni, Mo and Pb are
303 preferentially accumulated on the *coarse* fraction, Cu on PM 2.5 and finally Pt and Cr on PM1.
304 Published information about the size distribution of metals in urban atmosphere are not so extended;
305 although relatively high proportions of pollutants including transition metals are regularly found in
306 ultrafine PM (Sioutas et al., 2005). Considering their small size, ultrafine particles (and all that can
307 be conveyed by them) can also readily traverse biological membranes, facilitating systemic
308 distribution in the body and eventually revealing multiple local or systemic effects.
309
310 *3.2 Risk analysis*
311 The cumulative risk values from non-carcinogenic substances, THQ, calculated as in Eq. (1) for
312 each metals, for each PM principal fraction and for each receptors (adult and child) are shown in
313 **Figures 2**; these values ranged from 4.96×10^{-4} and 1.69 for adult while from 1.80×10^{-3} and 6.13 for
314 child. The cumulative risk values from carcinogenic substances, CR, calculated as in Eq. (2), for

each metals, for each PM principal fraction and for each receptors (adult and child) are shown in **Figures 3**; these values ranged from $3.71x10^{-8}$ and $1.04x10^{-5}$ for adult while from $5.84x10^{-8}$ and $9.46x10^{-6}$ for child. The non-carcinogenic risk was higher for children than for adults, in line with the fact that children are more sensitive to non-carcinogenic substances than adults **(Figure 2)** and the CR were comparable – in terms of order of magnitude – for adult and child. The highest THQ values for both adult and child were reported for Mn and Cd, in particular the *coarse* fraction was the more dangerous for Mn and the PM2.5 fraction for Cd.

Regarding the CR the highest value for both adult and child was reported for Cd without significant differences in PM fractions; the *coarse* fraction was the more dangerous for Co and As.

Given the observed differences it is fundamental to consider that any changes in the fractions amount in PM10 may correspond to a different metals intake and a different risk (carcinogenic and non-carcinogenic).

The results of the risk analysis showed that for the examined metals, the CR proved to be lower than 10^{-6} and the non-carcinogenic risk, THQ, lower than 1, in line with European guidelines with the exception of Cd, for which the total PM10 THQ were 1.69 and 6.13 while total PM10 CR were $1.04x10^{-5}$ and $9.46x10^{-6}$ for adult and child respectively), this result was clearly correlated to the high and remarkable Cd concentrations evaluated in this study probably due to the working activities related to the restoring of a building nearby the sampling site. We must also account the fact that exposure to metals occurs also via ingestion and dermal contact and, if these routes are considered, the estimated risks might be higher (Slezakova et al., 2013).

4. DISCUSSION

The composition of PM is very variable and depends on many different factors among which sources, climate and topography are only few examples. The chemical speciation of PM has been under study for many decades and the presence of heavy metals is known from past and recent literature (Chiari et al., 2006; Pey et al., 2010). Nevertheless there are few works that associate the

PM metals distribution to health effects stratified by dimensional particles size (Slezakova et al., 2013) by means of environmental-sanitary risk assessment. By our samplings metals appear to be variously distributed among all the fractions demonstrating their background presence. While the concentrations of all the sampled metals appear to be under control, consideration should be given to the significantly different presence of certain metals in the different PM fractions, which provides an indication related to the metals ability to penetrate into the respiratory tracts. Metals found in the finer fractions are potentially able to deeply penetrate into the bloodstream through the alveoli, to be carried far from respiratory tract.

In particular our results highlight Mn and Cd as the two metals reflecting the potential toxic or carcinogenic risks for human health. Mn is reported to be essential for the development of the brain, being the concentrations in human brain higher in adults (approximately 0.25 mg g^{-1} wet weight) than in infants, suggesting this metal as required for brain functions (Markesbery et al., 1984; Pomier-Layrargues et al., 1995; Takeda, 2003).When this metal is abnormally concentrated in the brain, especially in the basal ganglia, this results in neurological disorders similar to Parkinson's disease (Ono et al., 1995; Takeda, 2003), and neurotoxicity. Concerning Cd, due to its extremely protracted biological half-life (approximately 20–30 years in humans), low rate of excretion from the body and storage predominantly in soft tissues (primarily, liver and kidneys), this metal has a diversity of toxic effects including nephrotoxicity, carcinogenicity, teratogenicity and endocrine and reproductive toxicities. Current evidence suggests that exposure to Cd induces genomic instability through complex and multifactorial mechanisms. Most important seems to be Cd interaction with DNA mismatch repair mechanism (MMR) (Giaginis et al., 2006), and induction of apoptosis (Zarros, 2008).

Even if the levels of inhalation exposure to such metals associated with the PM may be considered too low to induce phenomena of toxicity according to the classical mechanisms, their presence - even in traces - could play an important role, being a chronic exposure factor, in the development of

biological mechanisms responsible for some of the recorded health effects on the local population, as confirmed by risk calculation.

In conclusion for all the examined metals, carcinogenic and non-carcinogenic, and for both children and adults, the inhalation of airborne PM10 in a urban site gave risk values about one order of magnitude lower than those indicated in guidelines with the exception of Cd (which reflects a "spot" situation); in this view the hazard quotient resulted higher for a child than for an adult while carcinogenic risk was similar. The absence of PM-metals risk in a urban site with high pollution levels (WHO, 2011) is concordant with the good reduction trend of metals in Europe (EEA, 2013) that however is a matter of concern for all the policies aimed at the protection of human health.

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FIGURES CAPTIONS

Figure 1: *Metals contribution (%) among the 3 main fractions of PM. Asterisks (*) provide an indication of statistically significant distribution ($p<0.01$, ANOVA test).*

Figure 2: *Cumulative risk values from non-carcinogenic substances – THQ. A) shows values for adults, B) shows values for children.THQ values are expressed as log-scale.*

Figure 3: *Cumulative risk values from carcinogenic substances – CR. A) shows values for adults, B) shows values for children. CR values are expressed as log-scale.*

Figure 1

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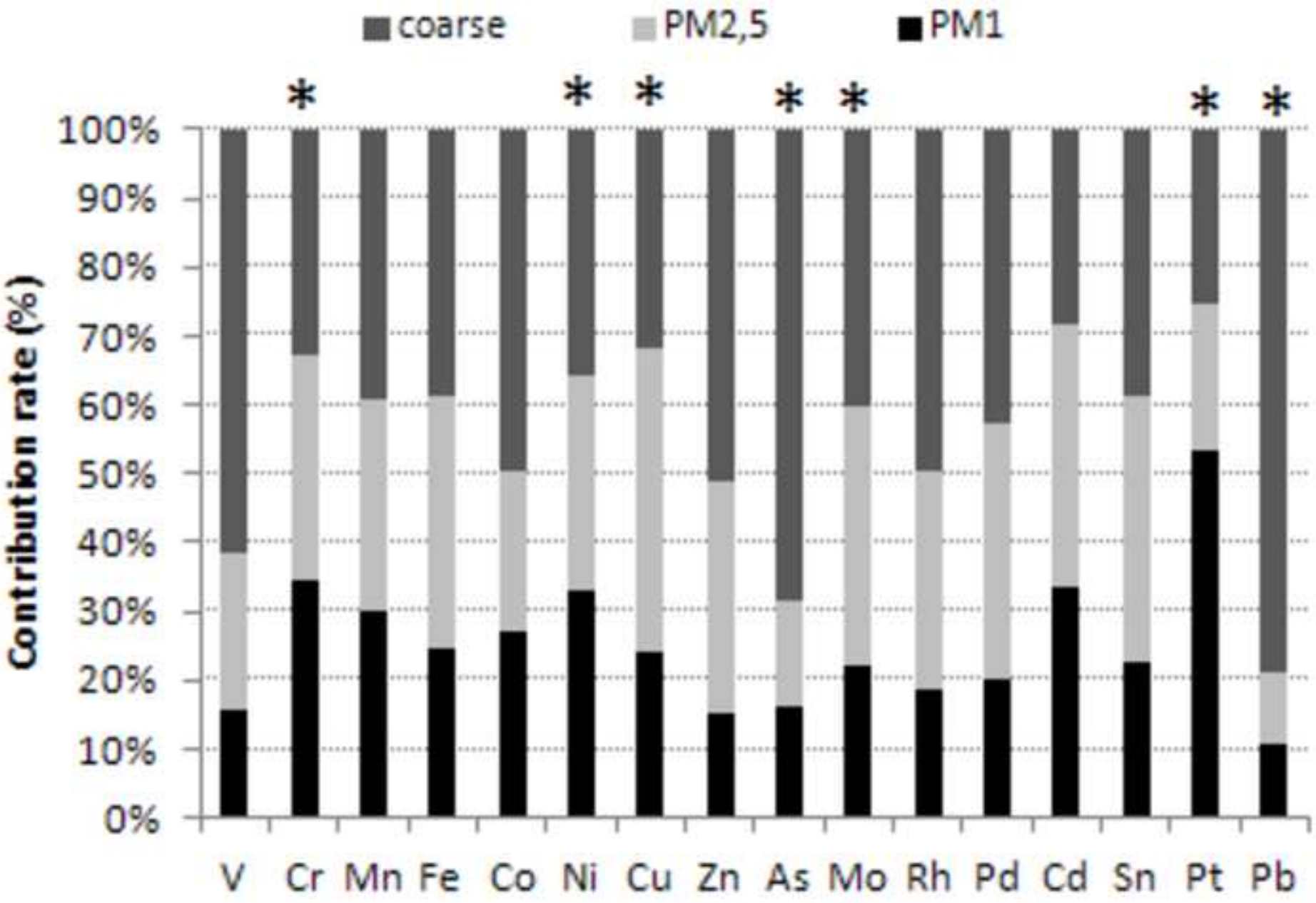

Figure 2

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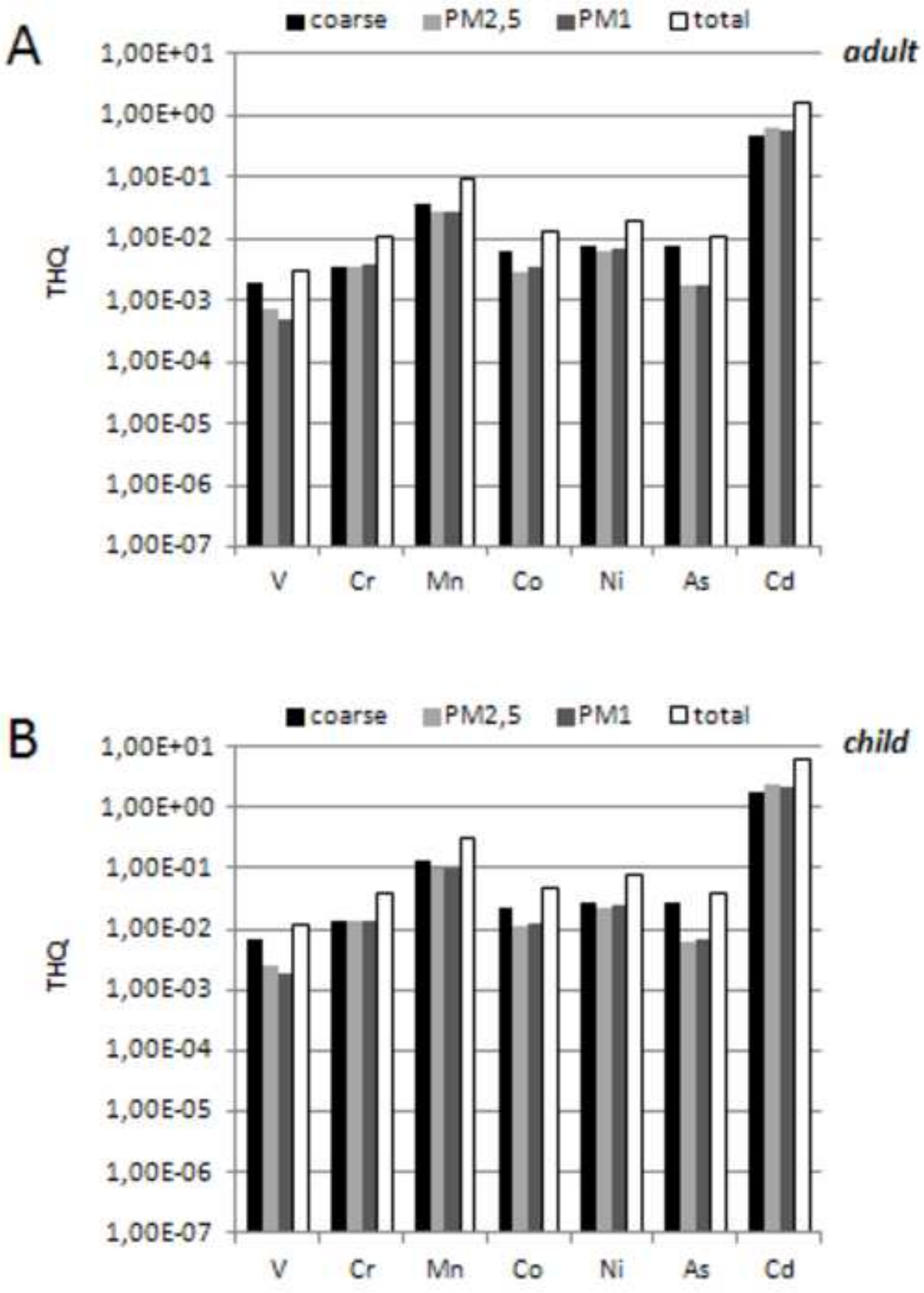

Figure 3

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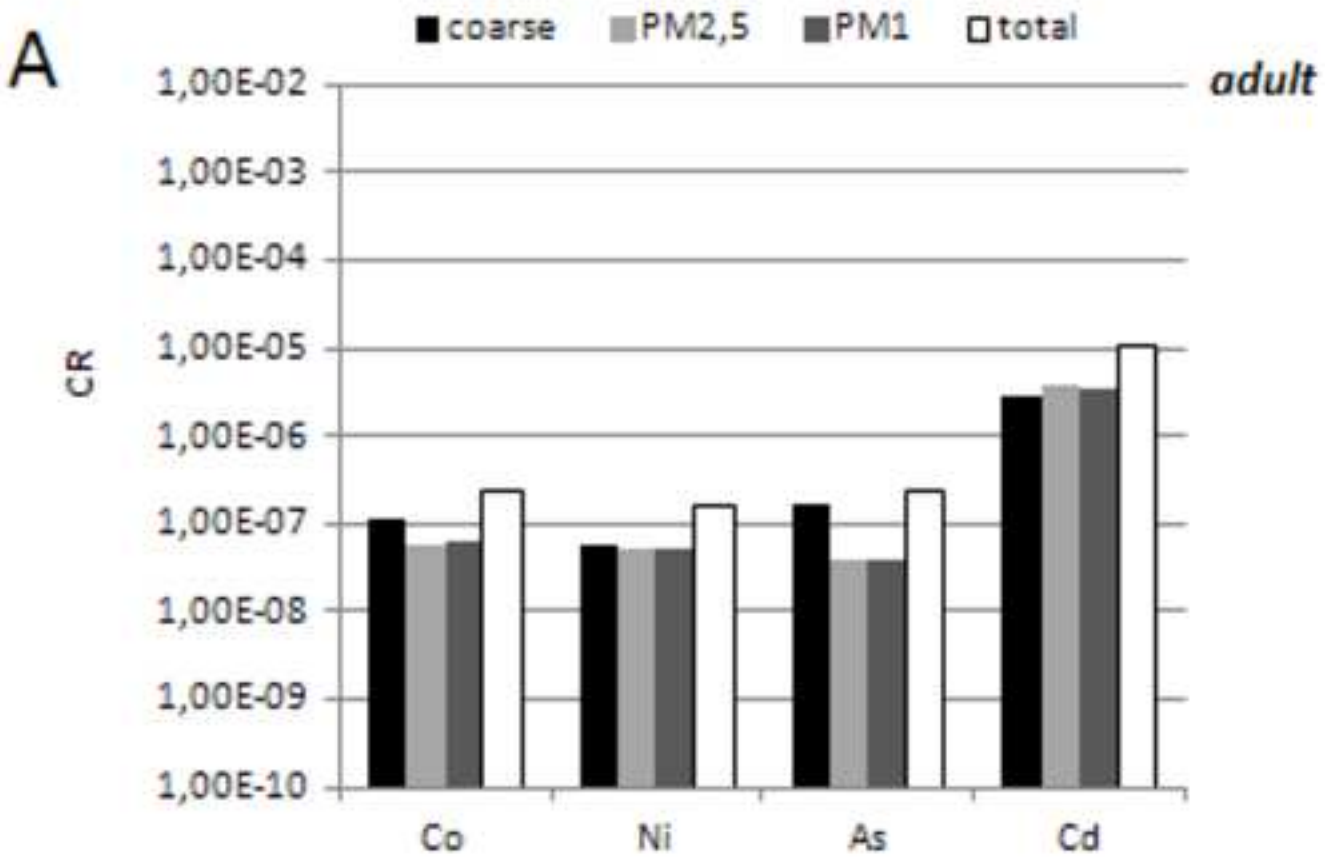

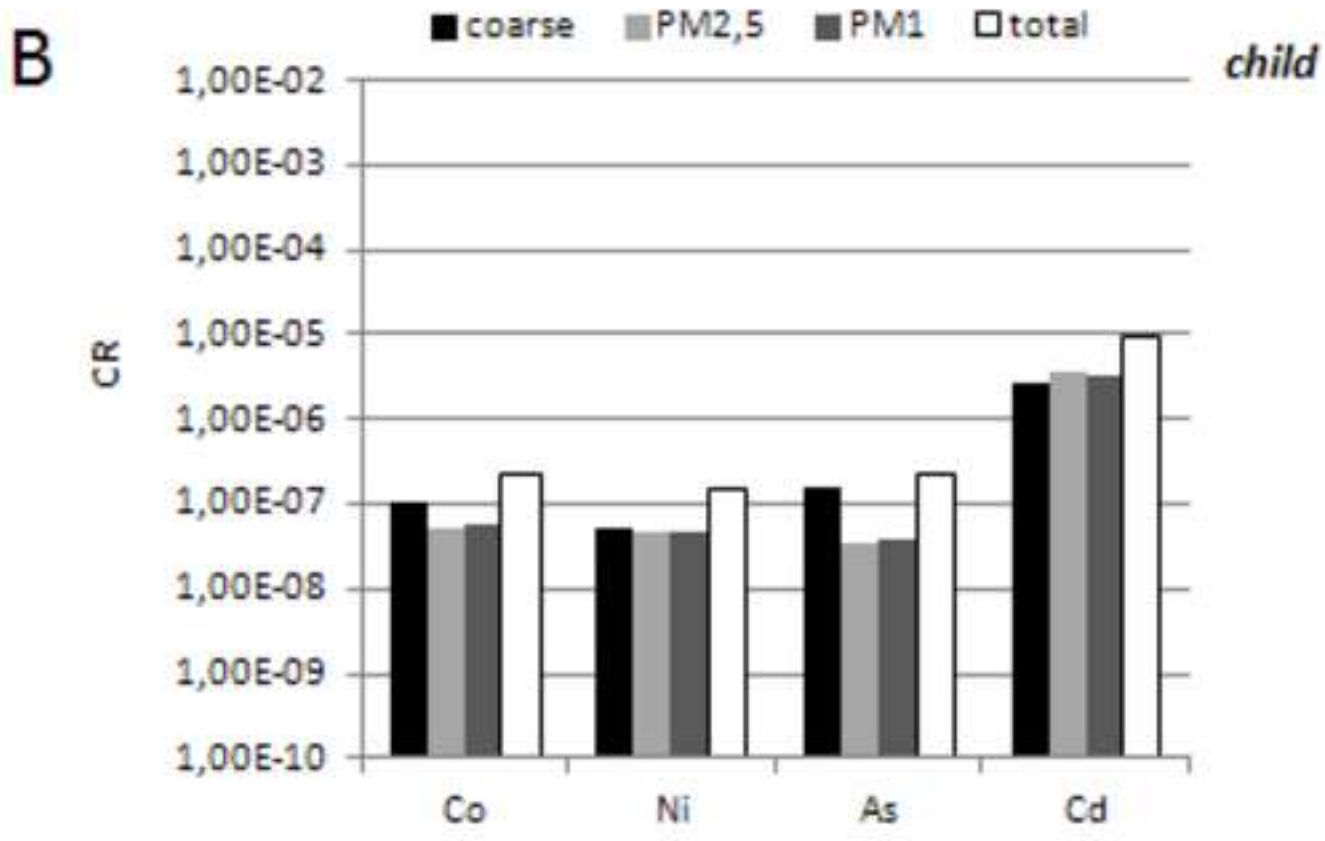

Table 1

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Table 1: *IUR and RfC data from USEPA database (USEPA 2013a); RfDi and CSF values were derived in according with Eq. (3) and Eq. (4) respectively, as recommended by USEPA methodology (USEPA 2013b).*

	CAS	IUR $[ug/m^3]^{-1}$	CSF $[mg/Kg\ day]^{-1}$	RfC $[mg/m^3]$	RfD*i* [mg/Kg day]
V	7440-62-2	-	-	1,00E-04	2,86E-05
Cr	7440-47-3	-	-	1,40E-04	4,00E-05
Mn	7439-96-5	-	-	5,00E-05	1,43E-05
Fe	7439-89-6	-	-	-	-
Co	7440-48-4	9,00E-03	3,15E+01	6,00E-06	1,71E-06
Ni	7440-02-0	2,60E-04	9,10E-01	9,00E-05	2,57E-05
Cu	7440-50-8	-	-	-	-
Zn	7440-66-6	-	-	-	-
As	7440-38-2	4,30E-03	1,51E+01	1,50E-05	4,29E-06
Mo	7439-98-7	-	-	-	-
Rh	7440-16-6	-	-	-	-
Pd	7440-05-3	-	-	-	-
Cd	7440-43-9	1,80E-03	6,30E+00	1,00E-05	2,86E-06
Sn	7440-31-5	-	-	-	-
Pt	7440-06-4	-	-	-	-
Pb	7439-92-1	-	-	-	-

Table 2. *List of parameters utilized for the calculation of the specific exposition rates. Standard default exposure factors, taking into account the "inhalation of contaminant" as exposure pathway in a "residential scenario" related to adults and children, male and female combined, for sedentary and light activity (USEPA 1989, USEPA 2009 – Part F and USEPA 2011).*

parameter	acronym	Unit of measurement	Numeric value	
			adult	child
inhalation rate	IR	m^3/h	0,9	0,7
Body weight	BW	Kg	70	15
Exposure time	ET	h/day	5	5
Exposure frequency	EF	day/year	350	350
Exposure duration	ED	years	24	6
Lifetime Average	ATc	days	70	70
Lifetime Average	ATn	days	=ED	=ED

*ATn = 365 days/years x ED; ATc = 365 days/years x 70 years

Table 3

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Table 3: *Metals abundance expressed as mean ng/m^3 and standard deviations (SD) among each of the size fractionated sampler stages are given. P values (ANOVA) are referred to the statistically significant dimensional-dependent distribution.*

Mean ng/m^3 (± SD)	FILTER 7 11,00 µm	FILTER 6 9,00 µm	FILTER 5 5,85 µm	FILTER 4 4,00 µm	FILTER 3 2,70 µm	FILTER 2 1,60 µm	FILTER 1 0,88 µm	FILTER 0 0,54 µm	TOTAL	*p*
V	0,377(±0,242)	0,253(±0,175)	0,149(±0,097)	0,127(±0,081)	0,200(±0,125)	0,133(±0,076)	0,093(±0,021)	0,137(±0,047)	1,469(±0,092)	-
Cr	0,413(±0,251)	0,477(±0,197)	0,573(±0,110)	0,883(±0,101)	1,247(±0,137)	1,100(±0,217)	1,150(±0,495)	1,350(±0,882)	7,193(±0,368)	0,049
Mn	1,790(±1,747)	2,233(±1,907)	1,820(±0,882)	2,330(±0,652)	3,753(±0,428)	2,613(±0,186)	2,320(±0,870)	3,980(±2,661)	20,840(±0,827)	-
Fe	8,315(±16,523)	14,045(±32,471)	215,567(±296,212)	203,673(±156,025)	264,373(±97,241)	156,080(±39,381)	112,000(±11,653)	172,633(±95,717)	1146,687(±92,934)	-
Co	0,077(±0,059)	0,039(±0,029)	0,033(±0,006)	0,030(±0,010)	0,040(±0,010)	0,043(±0,012)	0,040(±0,000)	0,057(±0,023)	0,359(±0,015)	-
Ni	1,260(±0,433)	0,673(±0,241)	0,577(±0,068)	0,563(±0,165)	0,960(±0,712)	1,703(±0,006)	1,470(±0,680)	1,337(±0,857)	8,543(±0,437)	-
Cu	0,717(±0,142)	0,813(±0,133)	1,513(±0,287)	2,943(±0,446)	4,753(±1,141)	3,610(±0,623)	2,057(±0,157)	2,450(±1,324)	18,857(±1,391)	< 0,0001
Zn	41,480(±43,890)	22,093(±17,926)	7,697(±2,441)	27,210(±17,893)	41,553(±29,052)	22,835(±21,821)	16,295(±43,001)	13,143(±5,930)	192,307(±12,375)	-
As	0,177(±0,106)	0,177(±0,122)	0,110(±0,087)	0,067(±0,012)	0,060(±0,017)	0,057(±0,015)	0,067(±0,012)	0,060(±0,036)	0,773(±0,052)	-
Mo	0,183(±0,021)	0,173(±0,038)	0,210(±0,053)	0,363(±0,060)	0,543(±0,235)	0,337(±0,067)	0,227(±0,006)	0,283(±0,146)	2,320(±0,124)	0,009
Rh	0,007(±0,000)	0,010(±0,000)	0,007(±0,006)	0,011(±0,009)	0,011(±0,008)	0,010(±0,010)	0,005(±0,005)	0,008(±0,004)	0,068(±0,002)	-
Pd	0,020(±0,014)	0,025(±0,007)	0,030(±0,000)	0,020(±0,014)	0,070(±0,078)	0,014(±0,010)	0,014(±0,009)	0,031(±0,010)	0,224(±0,018)	-
Cd	3,113(±1,497)	4,847(±5,078)	5,900(±4,345)	8,127(±4,963)	17,123(±13,136)	12,837(±10,260)	20,997(±33,050)	5,363(±6,235)	78,307(±6,493)	-
Sn	6,700(±6,915)	6,223(±4,844)	8,787(±7,103)	23,810(±24,458)	29,763(±25,990)	15,220(±10,722)	9,877(±9,136)	16,597(±20,651)	116,977(±8,517)	-
Pt	0,006(±0,005)	0,011(±0,008)	0,009(±0,002)	0,013(±0,006)	0,013(±0,006)	0,019(±0,012)	0,046(±0,039)	0,037(±0,021)	0,154(±0,014)	-
Pb	3,170(±1,696)	2,920(±1,658)	1,720(±0,828)	0,587(±0,121)	0,553(±0,040)	0,530(±0,262)	0,460(±0,321)	0,693(±0,475)	10,633(±1,135)	0,005